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<b>(21) International Application Number:</b> PCT/US96/12162 <b>(22) International Filing Date:</b> 23 July 1996 (23.07.96)  <b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ADAMOU, John, Edward [CA/US]; 318 Steeple Chase Drive, Exton, PA 19341 (US). ELSHOURBAGY, Nabil, Abd, Elsalam [US/US]; 1648 Bowtree Drive, West Chester, PA 19380 (US).  <b>(74) Agents:</b> HAN, William, T. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).		<b>(81) Designated States:</b> AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> CALCITONIN GENE-RELATED PEPTIDE RECEPTOR COMPONENT FACTOR (HOUDC44)			
<b>(57) Abstract</b>  Human CGRP-RCF polypeptides and DNA (RNA) encoding such CGRP-RCF and a procedure for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing such CGRP-RCF for the treatment of diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like. Antagonists against such CGRP-RCF and their use as a therapeutic to treat diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the CGRP-RCF and for detecting altered levels of the polypeptide in a host.			

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## CALCITONIN GENE-RELATED PEPTIDE RECEPTOR COMPONENT FACTOR (HOUDC44)

### FIELD OF THE INVENTION

5           This invention relates, in part, to newly identified polynucleotides and polypeptides; variants and derivatives of the polynucleotides and polypeptides; processes for making the polynucleotides and the polypeptides, and their variants and derivatives; agonists and antagonists of the polypeptides; and uses of the polynucleotides, polypeptides, variants, derivatives, agonists and antagonists. In particular, in these and in other regards, the invention relates to polynucleotides and polypeptides of human Calcitonin Gene-related Peptide Receptor Component Factor, hereinafter referred to as "CGRP-RCF".

### BACKGROUND OF THE INVENTION

15           Calcitonin gene related peptide (CGRP) is a 37 amino acid carboxyl-amidated neuropeptide secreted by the nerves of the central and peripheral nervous systems and exists as highly homologous  $\alpha$  or  $\beta$  isoforms in both human and rat (Amara, et al. *Nature* 298:240-244 (1982); Amara, et al. *Science* 229:1094-1097 (1985)).  $\alpha$ - and  $\beta$ -CGRP display very similar biological activities, including peripheral and cerebral vasodilation (Brain, et al., *Nature* 313:54-56 (1985)), cardiac acceleration, (Sigrist, et al., *Endocrinology* 119:381-389 (1986)), regulation of calcium metabolism (Grunditz, et al., *Endocrinology* 119:2313-2324 (1986)), reduction of intestinal motility (Fargeas, et al., *Peptides* 6:1167-1171(1985)), regulation of glucose metabolism (reduction of insulin secretion and insulin sensitivity) (Hermansen, et al., *Reg. Peptides* 27:149-157) (1990)), diminution of appetite (Molina, et al., *Diabetes* 39:260-265 (1990)) and reduction of growth hormone release (Tannenbaum, et al., *Endocrinology* 116:2685-2687 (1985)). The two CGRP peptides differ by three amino acids in humans and one amino acid in rats. The amino acid sequences of CGRP peptides are well conserved among species and can be considered as members of a family of peptides including the related peptides amylin (46% homology), salmon calcitonin (32% homology), and

adrenomedullin (24% homology). These peptides in general have N-terminal ring structures of 6-7 amino acids involving a disulfide bridge and an amidated C-terminal end (Muff, et al., *Eur J Endocrinol.* **133**:17-20 (1995); Goodman, et al., *Life Sci.* **38**:2169-2178 (1986); Poyner, D.R. *Pharmac.Ther.* **56**:23-51 (1992)).

- 5 CGRP peptides are localized predominantly in sensory afferent nerves and central neurons (Goodman, et al., *Life Sci.* **38**:2169-2178 (1986); Poyner, D.R. *Pharmac.Ther.* **56**:23-51 (1992)). When released from the cell, the peptides initiate their biological responses by binding to specific cell surface receptors which are predominantly coupled to the activation of adenylyl cyclase (Brain, et al., *Nature*
- 10 **313**:54-56 (1985); Kubota, et al., *Biochem Biophys Res Commun* **132**:88-94 (1985)). CGRP receptors have been identified and pharmacologically evaluated in several tissues and cells, including brain, cardiovascular, endothelial and smooth muscle (Poyner, D.R. *Pharmac.Ther.* **56**:23-51 (1992)). Multiple CGRP receptors have been observed, based on pharmacological properties. These receptors they are
- 15 divided into at least two subtypes and denoted as CGRP<sub>1</sub> and CGRP<sub>2</sub>, according to the classification of Dennis and colleagues (Dennis, et al., *Brain Res.* **539**:59-66 (1991)). CGRP (Molina, et al., *Diabetes* **39**:260-265 (1990); Tannenbaum, et al., *Endocrinology* **116**:2685-2687 (1985); Muff, et al., *Eur J Endocrinol.* **133**:17-20(1995); Goodman, et al., *Life Sci.* **38**:2169-2178 (1986); Poyner, D.R.,
- 20 *Pharmac.Ther.* **56**:23-51 (1992); Kubota, et al., *Biochem Biophys Res Commun* **132**:88-94 (1985); Dennis, et al., *Brain Res.* **539**:59-66 (1991); Adams, et al., *Science* **252**:1651-1656 (1991); Adams, et al., *Nature* **355**:632-634 (1992); Adams, et al., *Nature* **377**:3-174 (1995); Chang, et al., *Neuron* **11**:1187-1195 (1993); Fluhmann, et al., *Biochem. Biophys. Res. Comm.* **206**:341-347 (1995); Jelinek, et al.,
- 25 *Science* **259**:1614-1616 (1993); Kozak, M., *Proc. Natl. Acad. Sci. (USA)*, **92**:2662-2666 (1995); Aiyar, et al., *Mol. Cell. Bio.* **131**:75-86 (1994); Sambrook, et al., Molecular Cloning. Cold Spring Harbor Laboratory Press (1989); Nuovo, J. G., PCR in situ Hybridization: protocols and applications. Raven Press, New York (1992); Aiyar, et al. *Endocrinology* **129**:965-969 (1991); Lin, et al., *Science* **254**:1022-1024
- 30 (1991); Hirata, et al., *Biochem. Biophys. Res. Commun.* **151**:1113-1121 (1988); Nawa, et al., *Nature* **312**:729-734 (1984); Kozak, M. J., *Mol. Biol.* **196**: 947-950

(1987); Dohlman, et al., *Annu. Rev. Biochem.* **60**:653-688 (1991); Jackson, T., *Pharmacol. Ther.* **50**:425-442 (1991); Ishihara, et al., *EMBO J.*, **10**:1635-1641 (1991); Juppner, et al., *Science* **254**:1024-1026 (1991); Thorens, B., *Proc. Natl. Acad. Sci. U. S. A.*, **89**:8641-8645 (1992); van Valen, et al., *Neurosci. Lett.* **119**:195-198 (1990); Stangl, et al., *Endocrinology* **132**:744-750 (1993); Kitamura, et al., *FEBS Lett.*, **338**:306-310 (1994)), which lacks 7 N-terminal amino acid residues, is a selective antagonist of CGRP<sub>1</sub> receptors, whereas the linear analog of CGRP, diacetoamidomethyl cysteine CGRP ([Cys(ACM)<sub>2,7</sub>]CGRP), is a selective agonist of CGRP<sub>2</sub> receptors (Dennis, et al., *Brain Res.* **539**:59-66 (1991)).

As indicated above, CGRP has a plethora of functions in the body and is known to be the most potent vasodilator and neuromodulator. Although we recently reported the cDNA encoding the human CGRP-type I receptor (Aiyar et al., *Journal of Biological Chemistry* **271**:11325-11329 (1996)), we observed during our characterization studies that the receptor did not confer CGRP responsiveness in *Xenopus* oocytes, in transiently expressing COS cells, and in stably expressing *Baculovirus* and *Drosophila* cells. In contrast to these observations, we are able to confer reasonable levels of CGRP responsiveness in stably transfected human embryonic kidney 293 cells. These observations suggest the requirement of an additional human complementary factor for functional coupling of the CGRP receptor in all of these heterologous systems.

Recently, Luebke et al., (*Proc. Natl. Acad. Science*, **93**:3455-3460 (1996)) used an expression cloning strategy to isolate a guinea pig cDNA that encodes a protein (CGRP-receptor component protein) that confers CGRP responsiveness in oocytes. Utilizing this information and using the expressed sequence tag (EST) analysis (Adams, et al., *Science* **252**:1651-1656 (1991); Adams, et al., *Nature* **355**:632-634 (1992); Adams, et al., *Nature* **377**:3-174 (1995)) we identified the human homologue of the guinea pig CGRP-RCP. A full length cDNA clone is identified from a human adipocytes osteoclastoma cDNA library.

Although numerous groups have indicated the absolute requirement for complementary RCFs for functional signaling in heterologous systems for

several 7TM receptors (*JBC* 266:12560, 1991 and *FEBS Lett.* 291:208, 1991), none were able to successfully isolate the cDNA encoding these specific factors.

The identification and isolation of a complementary human factor necessary for expression of the CGRP type I receptor in heterologous systems, provides an opportunity to delineate more exactly or enhance the signaling pathways that are activated by the receptor. This also offers a novel approach to identify functionally the increased number of orphan cDNAs encoding suspected G-protein linked receptors whose ligands are still unknown.

Clearly, there is a need for factors necessary for correct signal transduction of CGRP receptors (or even other G-protein coupled receptors) and their roles in dysfunction and disease. There is a need, therefore, for identification and characterization of such factors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, such as, but not limited to, diabetes, migraine, pain and inflammation.

## SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide polypeptides, *inter alia*, that have been identified as novel CGRP-RCF by homology between the amino acid sequence set out in Figure 1 and known amino acid sequences of other proteins such as guinea pig CGRP-RCP described in by Luebke in the *Proc. Natl. Acad. Sci.* 93:3455-3460 (1996).

It is a further object of the invention, moreover, to provide polynucleotides that encode CGRP-RCF, particularly polynucleotides that encode the polypeptide herein designated CGRP-RCF.

In a particularly preferred embodiment of this aspect of the invention the polynucleotide comprises the region encoding human CGRP-RCF in the sequence set out in Figure 1.

In accordance with this aspect of the present invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressed by the human cDNA contained in ATCC Deposit No. 98105.

In accordance with this aspect of the invention there are provided isolated nucleic acid molecules encoding human CGRP-RCF, including mRNAs, cDNAs, genomic DNAs and, in further embodiments of this aspect of the invention, biologically, diagnostically, clinically or therapeutically useful variants, analogs or  
5 derivatives thereof, or fragments thereof, including fragments of the variants, analogs and derivatives.

Among the particularly preferred embodiments of this aspect of the invention are naturally occurring allelic variants of human CGRP-RCF.

It also is an object of the invention to provide CGRP-RCF polypeptides,  
10 particularly human CGRP-RCF polypeptides, that may be employed for therapeutic purposes, for example, to treat diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and  
15 the like.

In accordance with this aspect of the invention there are provided novel polypeptides of human origin referred to herein as CGRP-RCF as well as biologically, diagnostically or therapeutically useful fragments, variants and derivatives thereof, variants and derivatives of the fragments, and analogs of the foregoing.

20 Among the particularly preferred embodiments of this aspect of the invention are variants of human CGRP-RCF encoded by naturally occurring alleles of the human CGRP-RCF gene.

In accordance with another aspect of the present invention there are provided methods of screening for compounds which bind to and activate or inhibit activation of  
25 the CGRP receptor or CGRP/CGRP-RCF receptor polypeptides and for receptor ligands.

It is another object of the invention to provide a process for producing the aforementioned polypeptides, polypeptide fragments, variants and derivatives, fragments of the variants and derivatives, and analogs of the foregoing.

30 In a preferred embodiment of this aspect of the invention there are provided methods for producing the aforementioned CGRP-RCF polypeptides comprising

culturing host cells having expressibly incorporated therein an exogenously-derived human CGRP-RCF-encoding polynucleotide under conditions for expression of human CGRP-RCF in the host and then recovering the expressed polypeptide.

In accordance with another object the invention there are provided products,  
5 compositions, processes and methods that utilize the aforementioned polypeptides and polynucleotides for research, biological, clinical and therapeutic purposes, *inter alia*.

In accordance with certain preferred embodiments of this aspect of the invention, there are provided products, compositions and methods, *inter alia*, for, among other things: assessing CGRP-RCF expression in cells by determining CGRP-  
10 RCF polypeptides or CGRP-RCF-encoding mRNA; treating diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like, *in vitro*, *ex vivo* or *in vivo* by  
15 exposing cells to CGRP-RCF polypeptides or polynucleotides as disclosed herein; assaying genetic variation and aberrations, such as defects, in CGRP-RCF genes; and administering a CGRP-RCF polypeptide or polynucleotide to an organism to augment CGRP-RCF function or remediate CGRP-RCF dysfunction.

In accordance with still another embodiment of the present invention there is  
20 provided a process of using such activating compounds to stimulate the CGRP receptor polypeptide for the treatment of conditions related to the under-expression of the CGRP-RCF.

In accordance with another aspect of the present invention there is provided a process of using such inhibiting compounds for treating conditions associated with  
25 over-expression of the CGRP-RCF.

In accordance with yet another aspect of the present invention there is provided non-naturally occurring synthetic, isolated and/or recombinant CGRP-RCF polypeptides which are fragments, consensus fragments and/or sequences having conservative amino acid substitutions, of at least one domain of the CGRP-RCF of the  
30 present invention, such that the CGRP-RCF may bind to CGRP receptor ligands or to

CGRP receptor, or which may also modulate, quantitatively or qualitatively, CGRP receptor ligand binding.

In accordance with still another aspect of the present invention there are provided synthetic or recombinant CGRP-RCF polypeptides, conservative substitution  
5 and derivatives thereof, antibodies, anti-idiotypic antibodies, compositions and methods that can be useful as potential modulators of CGRP-RCF function, by binding to CGRP receptor ligands or CGRP receptor or modulating ligand binding, which may be used in diagnostic, therapeutic and/or research applications.

It is still another object of the present invention to provide synthetic, isolated or  
10 recombinant polypeptides which are designed to inhibit or mimic various CGRP-RCF or fragments thereof.

In accordance with certain preferred embodiments of this and other aspects of the invention there are provided probes that hybridize to human CGRP-RCF sequences.

15 In certain additional preferred embodiments of this aspect of the invention there are provided antibodies against CGRP-RCF polypeptides. In certain particularly preferred embodiments in this regard, the antibodies are highly selective for human CGRP-RCF.

In accordance with another aspect of the present invention, there are provided  
20 CGRP-RCF and CGRP-RCF/CGRP receptor agonists. Among preferred agonists are molecules that mimic CGRP-RCF, that bind to CGRP receptor molecules or CGRP-RCF, and that elicit or augment CGRP-RCF-induced responses. Also among preferred agonists are molecules that interact with CGRP-RCF or CGRP-RCF/CGRP-RCF receptor, or with other modulators of CGRP-RCF activities, and thereby potentiate or  
25 augment an effect of CGRP-RCF or more than one effect of CGRP-RCF.

In accordance with yet another aspect of the present invention, there are provided CGRP-RCF and CGRP-RCF/CGRP receptor antagonists. Among preferred antagonists are those which mimic CGRP-RCF so as to bind to CGRP receptor or CGRP-RCF but inhibit CGRP-RCF-induced response or more than one CGRP-RCF-  
30 induced response. Also among preferred antagonists are molecules that bind to or interact with CGRP-RCF or CGRP/CGRP-RCF receptor so as to inhibit an effect of

CGRP-RCF or more than one effect of CGRP-RCF or which prevent expression of CGRP-RCF.

In a further aspect of the invention there are provided compositions comprising a CGRP-RCF polynucleotide or a CGRP-RCF polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect of the invention, the compositions comprise a CGRP-RCF polynucleotide for expression of a CGRP-RCF polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of CGRP-RCF.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

## 20 BRIEF DESCRIPTION OF THE DRAWINGS

The following drawing depicts certain embodiments of the invention. It is illustrative only and do not limit the invention otherwise disclosed herein.

Figure 1 shows the nucleotide and deduced amino acid sequence of human CGRP-RCF.

25

## GLOSSARY

The following illustrative explanations are provided to facilitate understanding of certain terms used frequently herein, particularly in the examples. The explanations are provided as a convenience and are not limitative of the invention.

30 DIGESTION of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction

enzymes referred to herein are commercially available and their reaction conditions, cofactors and other requirements for use are known and routine to the skilled artisan.

For analytical purposes, typically, 1 µg of plasmid or DNA fragment is digested with about 2 units of enzyme in about 20 µl of reaction buffer. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 µg of DNA are digested with 20 to 250 units of enzyme in proportionately larger volumes.

Appropriate buffers and substrate amounts for particular restriction enzymes are described in standard laboratory manuals, such as those referenced below, and they are specified by commercial suppliers.

Incubation times of about 1 hour at 37°C are ordinarily used, but conditions may vary in accordance with standard procedures, the supplier's instructions and the particulars of the reaction. After digestion, reactions may be analyzed, and fragments may be purified by electrophoresis through an agarose or polyacrylamide gel, using well known methods that are routine for those skilled in the art.

GENETIC ELEMENT generally means a polynucleotide comprising a region that encodes a polypeptide or a region that regulates transcription or translation or other processes important to expression of the polypeptide in a host cell, or a polynucleotide comprising both a region that encodes a polypeptide and a region operably linked thereto that regulates expression.

Genetic elements may be comprised within a vector that replicates as an episomal element; that is, as a molecule physically independent of the host cell genome. They may be comprised within mini-chromosomes, such as those that arise during amplification of transfected DNA by methotrexate selection in eukaryotic cells. Genetic elements also may be comprised within a host cell genome; not in their natural state but, rather, following manipulation such as isolation, cloning and introduction into a host cell in the form of purified DNA or in a vector, among others.

ISOLATED means altered "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both.

For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living animal in its natural state is not "isolated," but the same

polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs.

- 5           As part of or following isolation, such polynucleotides can be joined to other polynucleotides, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated polynucleotides, alone or joined to other polynucleotides such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole
- 10   organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the polynucleotides and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of polynucleotides or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for
- 15   instance, which are not naturally occurring compositions, and, therein remain isolated polynucleotides or polypeptides within the meaning of that term as it is employed herein.

LIGATION refers to the process of forming phosphodiester bonds between two or more polynucleotides, which most often are double stranded DNAs.

- 20   Techniques for ligation are well known to the art and protocols for ligation are described in standard laboratory manuals and references, such as, for instance, Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989) and Maniatis et al., pg. 146, as cited below.

- 25           OLIGONUCLEOTIDE(S) refers to relatively short polynucleotides. Often the term refers to single-stranded deoxyribonucleotides, but it can refer as well to single-or double-stranded ribonucleotides, RNA:DNA hybrids and double-stranded DNAs, among others.

- Oligonucleotides, such as single-stranded DNA probe oligonucleotides, often
- 30   are synthesized by chemical methods, such as those implemented on automated oligonucleotide synthesizers. However, oligonucleotides can be made by a variety of

other methods, including in vitro recombinant DNA-mediated techniques and by expression of DNAs in cells and organisms.

Initially, chemically synthesized DNAs typically are obtained without a 5' phosphate. The 5' ends of such oligonucleotides are not substrates for phosphodiester bond formation by ligation reactions that employ DNA ligases typically used to form recombinant DNA molecules. Where ligation of such oligonucleotides is desired, a phosphate can be added by standard techniques, such as those that employ a kinase and ATP.

The 3' end of a chemically synthesized oligonucleotide generally has a free hydroxyl group and, in the presence of a ligase, such as T4 DNA ligase, readily will form a phosphodiester bond with a 5' phosphate of another polynucleotide, such as another oligonucleotide. As is well known, this reaction can be prevented selectively, where desired, by removing the 5' phosphates of the other polynucleotide(s) prior to ligation.

PLASMIDS generally are designated herein by a lower case p preceded and/or followed by capital letters and/or numbers, in accordance with standard naming conventions that are familiar to those of skill in the art. Starting plasmids disclosed herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids by routine application of well known, published procedures. Many plasmids and other cloning and expression vectors that can be used in accordance with the present invention are well known and readily available to those of skill in the art. Moreover, those of skill readily may construct any number of other plasmids suitable for use in the invention. The properties, construction and use of such plasmids, as well as other vectors, in the present invention will be readily apparent to those of skill from the present disclosure.

POLYNUCLEOTIDE(S) generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, polynucleotides as used herein refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be

single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions.

In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be  
5 from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide.

As used herein, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones  
10 modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein.

It will be appreciated that a great variety of modifications have been made to  
15 DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia.

20 POLYPEPTIDES, as used herein, includes all polypeptides as described below. The basic structure of polypeptides is well known and has been described in innumerable textbooks and other publications in the art. In this context, the term is used herein to refer to any peptide or protein comprising two or more amino acids joined to each other in a linear chain by peptide bonds. As used herein, the term refers  
25 to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types.

It will be appreciated that polypeptides often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids,  
30 and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes, such as processing and other post-

translational modifications, but also by chemical modification techniques which are well known to the art. Even the common modifications that occur naturally in polypeptides are too numerous to list exhaustively here, but they are well described in basic texts and in more detailed monographs, as well as in a voluminous research

5 literature, and they are well known to those of skill in the art.

Among the known modifications which may be present in polypeptides of the present are, to name an illustrative few, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a  
10 lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation,  
15 prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Such modifications are well known to those of skill and have been described in great detail in the scientific literature. Several particularly common modifications, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid  
20 residues, hydroxylation and ADP-ribosylation, for instance, are described in most basic texts, such as, for instance PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., Posttranslational Protein Modifications: Perspectives and  
25 Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., Analysis for protein modifications and nonprotein cofactors, Meth. Enzymol. 182: 626-646 (1990) and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62 (1992).

30 It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result

of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translation natural process  
5 and by entirely synthetic methods, as well.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such  
10 modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in *E. coli*, prior to proteolytic processing, almost invariably will be N-formylmethionine.

The modifications that occur in a polypeptide often will be a function of how it is made. For polypeptides made by expressing a cloned gene in a host, for instance,  
15 the nature and extent of the modifications in large part will be determined by the host cell posttranslational modification capacity and the modification signals present in the polypeptide amino acid sequence. For instance, as is well known, glycosylation often does not occur in bacterial hosts such as *E. coli*. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a  
20 eukaryotic cell. Insect cell often carry out the same posttranslational glycosylations as mammalian cells and, for this reason, insect cell expression systems have been developed to express efficiently mammalian proteins having native patterns of glycosylation, inter alia. Similar considerations apply to other modifications.

It will be appreciated that the same type of modification may be present in the  
25 same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

30 **VARIANT(S)** of polynucleotides or polypeptides, as the term is used herein, are polynucleotides or polypeptides that differ from a reference polynucleotide or

polypeptide, respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail.

- (1) A polynucleotide that differs in nucleotide sequence from another, reference polynucleotide. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

As noted below, changes in the nucleotide sequence of the variant may be silent. That is, they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference. Also as noted below, changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below.

- (2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical.

A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

FUSION PROTEINS: EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, sIL5- $\alpha$  has been fused with Fc portions for the purpose of high-throughput

screening assays to identify antagonists of hIL-5. See, Bennett et al., *Journal of Molecular Recognition*, 8:52-58 (1995) and Johanson et al., *The Journal of Biological Chemistry*, 270, No. 16, 9459-9471 (1995).

Thus, this invention also relates to genetically engineered soluble fusion  
5 proteins comprised from CGRP-RCF, or a portion thereof, and of various portions of  
the constant regions of heavy or light chains of immunoglobulins of various subclass  
(IgG, IgM, IgA, IgE). Preferred as immunoglobulin is the constant part of the heavy  
chain of human IgG, particularly IgG1, where fusion takes place at the hinge region.  
In a particular embodiment, the Fc part can be removed in a simple way by a cleavage  
10 sequence which is also incorporated and can be cleaved with factor Xa. Furthermore,  
this invention relates to processes for the preparation of these fusion by genetic  
engineering, and to the use thereof for diagnosis and therapy. An yet further aspect of  
the invention also relates to polynucleotide encoding such fusion proteins.

**BINDING MOLECULES** (or otherwise called **INTERACTION**  
15 **MOLECULES** or **RECEPTOR COMPONENT FACTORS**) refer to molecules other  
than CGRP receptor ligands or CGRP receptor that specifically bind to or interact with  
polypeptides of the present invention. Such binding molecules are a part of the present  
invention. **BINDING MOLECULES** also may be non-naturally occurring, such as  
antibodies and antibody-derived reagents that bind specifically to polypeptides of the  
20 invention.

**CGRP-RCF/ CGRP RECEPTOR SYSTEM** or **CGRP-RCF/ CGRP**  
**RECEPTOR** or simply **RECEPTOR SYSTEM** as used herein refers to a complex  
formed between CGRP receptor and CGRP-RCF.

## 25 DESCRIPTION OF THE INVENTION

The present invention relates to novel CGRP-RCF polypeptides and  
polynucleotides, among other things, as described in greater detail below. In  
particular, the invention relates to polypeptides and polynucleotides of a novel human  
CGRP-RCF, which is related by amino acid sequence homology to guinea pig CGRP-  
30 RCF polypeptide. The invention relates especially to CGRP-RCF having the  
nucleotide and amino acid sequences set out in Figure 1, and to the CGRP-RCF

nucleotide and amino acid sequences of the human cDNA in ATCC Deposit No. 98105, which is herein referred to as "the deposited clone" or as the "cDNA of the deposited clone." It will be appreciated that the nucleotide and amino acid sequences set out in Figure 1 are obtained by sequencing the cDNA of the deposited clone.

- 5 Hence, the sequence of the deposited clone is controlling as to any discrepancies between the two and any reference to the sequences of Figure 1 include reference to the sequence of the human cDNA of the deposited clone.

### Polynucleotides

- 10 In accordance with one aspect of the present invention, there are provided isolated polynucleotides which encode the CGRP-RCF polypeptide having the deduced amino acid sequence of Figure 1.

- Using the information provided herein, such as the polynucleotide sequence set out in Figure 1, a polynucleotide of the present invention encoding  
15 human CGRP-RCF polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA from human adipocytes from osteoclastoma as starting material. Illustrative of the invention, the polynucleotide set out in Figure 1 is discovered in a cDNA library derived from human adipocytes from osteoclastoma using the expressed sequence tag  
20 (EST) analysis (Adams, et al., *Science* 252:1651-1656 (1991); Adams, et al., *Nature* 355:632-634 (1992); Adams, et al., *Nature* 377 Supp, 3-174 (1995)).

- Human CGRP-RCF of the invention is structurally related to the guinea pig CGRP-RCP, as shown by the results of sequencing the cDNA encoding human CGRP-RCF in the deposited clone. The cDNA sequence thus obtained is set out in  
25 Figure 1. SEQ ID NO: 1. It contains an open reading frame encoding a protein of 148 amino acid residues with a deduced molecular weight of about 17.7 kDa. CGRP-RCF of Figure 1 has about 88% identity and about 94% similarity with the amino acid sequence of guinea pig CGRP-RCP.

- Polynucleotides of the present invention may be in the form of RNA, such as  
30 mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination

thereof. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The coding sequence which encodes the polypeptide may be identical to the  
5 coding sequence of the polynucleotide shown in Figure 1. SEQ ID NO: 1. It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes the polypeptide of the DNA of Figure 1.

Polynucleotides of the present invention which encode the polypeptide of  
Figure 1 may include, but are not limited to the coding sequence for the mature  
10 polypeptide, by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and  
15 non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a  
20 marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in the pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci., USA* 86:821-824 (1989), for instance,  
25 hexa-histidine provides for convenient purification of the fusion protein. The HA tag corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson et al., *Cell* 37: 767 (1984), for instance.

In accordance with the foregoing, the term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides which include a sequence  
30 encoding a polypeptide of the present invention, particularly the human CGRP-RCF having the amino acid sequence set out in Figure 1. The term also encompasses

polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, interrupted by introns) together with additional regions, that also may contain coding and/or non-coding sequences.

The present invention further relates to variants of the herein above described  
5 polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1. A variant of the polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis  
10 techniques, including those applied to polynucleotides, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may  
15 produce conservative or non-conservative amino acid substitutions, deletions or additions.

Among the particularly preferred embodiments of the invention in this regard are polynucleotides encoding polypeptides having the amino acid sequence of CGRP-RCF set out in Figure 1; variants, analogs, derivatives and fragments thereof, and  
20 fragments of the variants, analogs and derivatives.

Further particularly preferred in this regard are polynucleotides encoding CGRP-RCF variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, which have the amino acid sequence of the CGRP-RCF polypeptide of Figure 1 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino  
25 acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the CGRP-RCF. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polynucleotides encoding polypeptides having the amino acid sequence of Figure 1, without  
30 substitutions.

Further preferred embodiments of the invention are polynucleotides that are at least 70% identical to a polynucleotide encoding the CGRP-RCF polypeptide having the amino acid sequence set out in Figure 1, and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical to a polynucleotide encoding the CGRP-RCF polypeptide of the human cDNA of the deposited clone and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Particularly preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences.

As discussed additionally herein regarding polynucleotide assays of the invention, for instance, polynucleotides of the invention as discussed above, may be used as a hybridization probe for cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding CGRP-RCF and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the human CGRP-RCF gene. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 30 bases and may have at least 50 bases. Particularly preferred probes will have at least 30 bases and will have 50 bases or less.

For example, the coding region of the CGRP-RCF gene may be isolated by screening using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to human disease, as further discussed herein relating to polynucleotide assays, *inter alia*.

The polynucleotides may encode a polypeptide which is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may facilitate protein trafficking, may prolong or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in situ*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In sum, a polynucleotide of the present invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences which are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

#### Deposited materials

A deposit containing a human CGRP-RCF cDNA has been deposited with the American Type Culture Collection, as noted above. Also as noted above, the human cDNA deposit is referred to herein as "the deposited clone" or as "the cDNA of the deposited clone."

5       The deposited clone is deposited with the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, USA, on July 17, 1996, and assigned ATCC Deposit No. 98105 (*Escherichia coli* pHOUDC44/SolR).

      The deposited material is a pBluescript SK (-) plasmid (Stratagene, La Jolla, CA)\*] that contains the full length CGRP-RCF cDNA, referred to as pHOUDC44  
10   upon deposit.

      The deposit has been made under the terms of the Budapest Treaty on the international recognition of the deposit of micro-organisms for purposes of patent procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposit is provided merely as  
15   convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

      The sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

20       A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

### Polypeptides

      The present invention further relates to a human CGRP-RCF polypeptide  
25   which has the deduced amino acid sequence of Figure 1. SEQ ID NO:2.

      The invention also relates to fragments, analogs and derivatives of these polypeptides. The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1, means a polypeptide which retains essentially the same biological function or activity as such polypeptide, i.e. functions as a CGRP-RCF, or  
30   retains the ability to bind the CGRP receptor ligand or the CGRP receptor even though the polypeptide does not function as a CGRP-RCF, for example, a soluble form of

CGRP-RCF. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide. In certain preferred embodiments it is a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Among the particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of CGRP-RCF set out in Figure 1, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments. Alternatively, particularly preferred embodiments of the invention in this regard are polypeptides having the amino acid sequence of the CGRP-RCF, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues

Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of the CGRP-RCF polypeptide of Figure 1, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the CGRP-RCF. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having the amino acid sequence of Figure 1 without substitutions.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 80% identity to the polypeptide of SEQ ID NO:2 and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of SEQ ID NO:2 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. Moreover, also known in the art is "identity" which means the degree of sequence relatedness between two polypeptides or two polynucleotides sequences as determined by the identity of the match between two strings of such sequences. Both identity and similarity can be readily calculated (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994;

*Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity and similarity between two polynucleotide or polypeptide sequences, the terms "identity" and "similarity" are well known to skilled artisans (*Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to disclosed in *Guide to Huge Computers*, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the two sequences tested. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215:403 (1990)).

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

#### **Fragments**

Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of CGRP-RCF, most particularly fragments of the CGRP-RCF having the amino acid set out in Figure 1, and fragments of variants and derivatives of the CGRP-RCF of Figure 1.

In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned CGRP-RCF polypeptides and variants or derivatives thereof.

Such fragments may be "free-standing," i.e., not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the presently discussed fragments most preferably form a single continuous region. However, several fragments may be comprised within a single larger polypeptide. For instance, certain preferred embodiments relate to a fragment of a CGRP-RCF polypeptide of the present comprised within a precursor polypeptide designed for expression in a host and having heterologous pre and pro-polypeptide regions fused to the amino terminus of the CGRP-RCF fragment and an additional region fused to the carboxyl terminus of the fragment. Therefore, fragments in one aspect of the meaning intended herein, refers to the portion or portions of a fusion polypeptide or fusion protein derived from CGRP-RCF.

As representative examples of polypeptide fragments of the invention, there may be mentioned those which have from about 5-15, 10-20, 15-40, 30-55, 41-75, 41-80, 41-90, 50-100, 75-100, 90-115, 100-125, and 110-113 amino acids long.

In this context about includes the particularly recited range and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes. For instance, about 40-90 amino acids in this context means a polypeptide fragment of 40 plus or minus several, a few, 5, 4, 3, 2 or 1 amino acids to 90 plus or minus several a few, 5, 4, 3, 2 or 1 amino acid residues, i.e., ranges as broad as 40 minus several amino acids to 90 plus several amino acids to as narrow as 40 plus several amino acids to 90 minus several amino acids.

Highly preferred in this regard are the recited ranges plus or minus as many as 5 amino acids at either or at both extremes. Particularly highly preferred are the recited ranges plus or minus as many as 3 amino acids at either or at both the recited extremes. Especially particularly highly preferred are ranges plus or minus 1 amino acid at either or at both extremes or the recited ranges with no additions or deletions. Most highly preferred of all in this regard are fragments from about 5-15, 10-20, 15-

40, 30-55, 41-75, 41-80, 41-90, 50-100, 75-100, 90-115, 100-125, and 110-113 amino acids long.

Among especially preferred fragments of the invention are truncation mutants of CGRP-RCF. Truncation mutants include CGRP-RCF polypeptides having the amino acid sequence of Figure 1, or of variants or derivatives thereof, except for deletion of a continuous series of residues (that is, a continuous region, part or portion) that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or, as in double truncation mutants, deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Fragments having the size ranges set out above also are preferred embodiments of truncation fragments, which are especially preferred among fragments generally.

Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of CGRP-RCF. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of CGRP-RCF.

Among highly preferred fragments in this regard are those that comprise regions of CGRP-RCF that combine several structural features, such as several of the features set out above. In this regard, the regions defined by the residues about 10 to about 20, about 40 to about 50, about 70 to about 90 and about 100 to about 113 of Figure 1, which all are characterized by amino acid compositions highly characteristic of turn-regions, hydrophilic regions, flexible-regions, surface-forming regions, and high antigenic index-regions, are especially highly preferred regions. Such regions may be comprised within a larger polypeptide or may be by themselves a preferred fragment of the present invention, as discussed above. It will be appreciated that the term "about" as used in this paragraph has the meaning set out above regarding fragments in general.

Further preferred regions are those that mediate activities of CGRP-RCF.

Most highly preferred in this regard are fragments that have a chemical, biological or other activity of CGRP-RCF, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Highly preferred in this regard are  
5 fragments that contain regions that are homologs in sequence, or in position, or in both sequence and to active regions of related polypeptides, such as the related polypeptides of guinea pig CGRP-RCP. Among particularly preferred fragments in these regards are truncation mutants, as discussed above.

It will be appreciated that the invention also relates to, among others,  
10 polynucleotides encoding the aforementioned fragments, polynucleotides that hybridize to polynucleotides encoding the fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode the fragments. In these regards, preferred polynucleotides are those that correspondent to the preferred fragments, as discussed above.

15

#### **Vectors, host cells, expression**

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant  
20 techniques.

Host cells can be genetically engineered to incorporate polynucleotides and express polypeptides of the present invention. For instance, polynucleotides may be introduced into host cells using well known techniques of infection, transduction, transfection, transvection and transformation. The polynucleotides may be introduced  
25 alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

Thus, for instance, polynucleotides of the invention may be transfected into host cells with another, separate, polynucleotide encoding a selectable marker, using  
30 standard techniques for co-transfection and selection in, for instance, mammalian cells.

In this case the polynucleotides generally will be stably incorporated into the host cell genome.

Alternatively, the polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. The vector construct may be introduced  
5 into host cells by the aforementioned techniques. Generally, a plasmid vector is introduced as DNA in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. Electroporation also may be used to introduce polynucleotides into a host. If the vector is a virus, it may be packaged in vitro or introduced into a packaging cell and the packaged virus may be transduced into cells.  
10 A wide variety of techniques suitable for making polynucleotides and for introducing polynucleotides into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length in Sambrook et al. cited above, which is illustrative of the many laboratory manuals that detail these techniques.

15 In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. The vectors, in the case of phage and viral vectors also may be and  
20 preferably are introduced into cells as packaged or encapsidated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of  
25 polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

30 In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only

in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including  
5 constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

The engineered host cells can be cultured in conventional nutrient media, which may be modified as appropriate for, inter alia, activating promoters, selecting transformants or amplifying genes. Culture conditions, such as temperature, pH and  
10 the like, previously used with the host cell selected for expression generally will be suitable for expression of polypeptides of the present invention as will be apparent to those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors  
15 e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as  
20 cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a  
25 variety of well-known and routine techniques. In general, a DNA sequence for expression is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction endonucleases and then joining the restriction fragments together using T4 DNA ligase. Procedures for restriction and ligation that can be used to this end are well known and routine to those of skill.  
30 Suitable procedures in this regard, and for constructing expression vectors using

alternative techniques, which also are well known and routine to those skilled in the art, are set forth in great detail in Sambrook et al. cited elsewhere herein.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to  
5 direct mRNA transcription. Representatives of such promoters include the phage  
lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40 early and late  
promoters and promoters of retroviral LTRs, to name just a few of the well-known  
promoters. It will be understood that numerous promoters not mentioned are suitable  
for use in this aspect of the invention are well known and readily may be employed by  
10 those of skill in the manner illustrated by the discussion and the examples herein.

In general, expression constructs will contain sites for transcription initiation  
and termination, and, in the transcribed region, a ribosome binding site for translation.  
The coding portion of the mature transcripts expressed by the constructs will include a  
translation initiating AUG at the beginning and a termination codon appropriately  
15 positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as  
engender expression. Generally, in accordance with many commonly practiced  
procedures, such regions will operate by controlling transcription, such as repressor  
binding sites and enhancers, among others.

20 Vectors for propagation and expression generally will include selectable  
markers. Such markers also may be suitable for amplification or the vectors may  
contain additional markers for this purpose. In this regard, the expression vectors  
preferably contain one or more selectable marker genes to provide a phenotypic trait  
for selection of transformed host cells. Preferred markers include dihydrofolate  
25 reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or  
ampicillin resistance genes for culturing E. coli and other bacteria.

The vector containing the appropriate DNA sequence as described elsewhere  
herein, as well as an appropriate promoter, and other appropriate control sequences,  
may be introduced into an appropriate host using a variety of well known techniques  
30 suitable to expression therein of a desired polypeptide. Representative examples of  
appropriate hosts include bacterial cells, such as E. coli, Streptomyces and Salmonella

typhimurium cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts for of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for  
5 expressing a polypeptides in accordance with this aspect of the present invention.

More particularly, the present invention also includes recombinant constructs, such as expression constructs, comprising one or more of the sequences described above. The constructs comprise a vector, such as a plasmid or viral vector, into which  
10 such a sequence of the invention has been inserted. The sequence may be inserted in a forward or reverse orientation. In certain preferred embodiments in this regard, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and there are many commercially available vectors suitable for use in the present invention.

15 The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred  
20 eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid  
25 or vector suitable for, for example, introduction, maintenance, propagation or expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a  
30 chloramphenicol acetyl transferase ("CAT") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment

that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the cat gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available. Two such vectors  
5 are pKK232-8 and pCM7. Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known bacterial promoters suitable for expression of polynucleotides  
10 and polypeptides in accordance with the present invention are the E. coli lacI and lacZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR, PL promoters and the trp promoter.

Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late  
15 SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction,  
20 introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be  
25 a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al. BASIC  
30 METHODS IN MOLECULAR BIOLOGY, (1986).

Constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

5 Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory  
10 Press, Cold Spring Harbor, N.Y. (1989).

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector. Among suitable promoters are those derived from  
15 the genes that encode glycolytic enzymes such as 3-phosphoglycerate kinase ("PGK"), a-factor, acid phosphatase, and heat shock proteins, among others. Selectable markers include the ampicillin resistance gene of *E. coli* and the *trp1* gene of *S. cerevisiae*.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the  
20 vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus  
25 enhancers.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located  
30 appropriately 5' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will

be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiating AUG. Also, generally, there will be a translation stop codon at the end of the polypeptide and there will be a polyadenylation signal and a transcription termination signal appropriately disposed at the 3' end of the transcribed region.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

Suitable prokaryotic hosts for propagation, maintenance or expression of polynucleotides and polypeptides in accordance with the invention include *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium*. Various species of *Pseudomonas*, *Streptomyces*, and *Staphylococcus* are suitable hosts in this regard. Moreover, many other hosts also known to those of skill may be employed in this regard.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, where the selected promoter is inducible it is induced by appropriate means (e.g., temperature shift or exposure to chemical inducer) and cells are cultured for an additional period.

- 5        Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

- 10       Various mammalian cell culture systems can be employed for expression, as well. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblast, described in Gluzman et al., Cell 23: 175 (1981). Other cell lines capable of expressing a compatible vector include for example, the C127, 3T3, CHO, HeLa, human kidney 293 and BHK cell lines.

- 15       Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are necessary for expression. In certain preferred embodiments in this regard DNA sequences derived from the SV40 splice sites, and the SV40 polyadenylation sites are used for required non-transcribed genetic elements of these types.

- 20       The CGRP-RCF polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and
- 30       or purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed  
5 in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

CGRP-RCF polynucleotides and polypeptides may be used in accordance with  
10 the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of CGRP-RCF. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are illustrated further by the following discussion.

#### 15 Polynucleotide assays

This invention is also related to the use of the CGRP-RCF polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of CGRP-RCF associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a  
20 disease which results from under-expression over-expression or altered expression of CGRP-RCF. Individuals carrying mutations in the human CGRP-RCF gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be  
25 amplified enzymatically by using PCR prior to analysis. PCR (Saiki et al., Nature, 324: 163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding CGRP-RCF can be used to identify and analyze CGRP-RCF expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in  
30 comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled CGRP-RCF RNA or alternatively, radiolabeled

CGRP-RCF antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations  
5 also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence  
10 determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized  
15 by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., *Science* 230: 1242 (1985)).

20 Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., *Proc. Natl. Acad. Sci., USA*, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or  
25 the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In accordance with a further aspect of the invention, there is provided a process for determining or diagnosing diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension,  
30 angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and

the like or a susceptibility to the foregoing diseases. Thus, a mutation in CGRP-RCF indicates a susceptibility to treat diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Thus, for example, the assay may be employed to determine a mutation in a human CGRP-RCF protein as herein described, such as a deletion, truncation, insertion, frame shift, etc., with such mutation being indicative of a susceptibility to the foregoing diseases.

A mutation may be ascertained for example, by a DNA sequencing assay. Tissue samples, including but not limited to blood samples are obtained from a human patient. The samples are processed by methods known in the art to capture the RNA. First strand cDNA is synthesized from the RNA samples by adding an oligonucleotide primer consisting of polythymidine residues which hybridize to the polyadenosine stretch present on the mRNA's. Reverse transcriptase and deoxynucleotides are added to allow synthesis of the first strand cDNA. Primer sequences are synthesized based on the DNA sequence of the protein of the invention. The primer sequence is generally comprised of at least 15 consecutive bases, and may contain at least 30 or even 50 consecutive bases.

Thus, the detection of a specific DNA sequence and/or quantitation of the level of the sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA. The invention provides a process for diagnosing diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like, comprising determining from a sample derived from a patient an abnormally decreased or increased level of expression of polynucleotide having the sequence of Figure 1 (SEQ ID NO: 1).

Decreased or increased expression of polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

- 5           In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

- Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location. As an example of how this is performed, CGRP-RCF DNA is digested and purified with
- 10   QIAEX II DNA purification kit (QIAGEN, Inc., Chatsworth, CA) and ligated to Super CosI cosmid vector (STRATAGENE, La Jolla, CA). DNA is purified using Qiagen Plasmid Purification Kit (QIAGEN Inc., Chatsworth, CA) and 1 mg is labeled by nick translation in the presence of Biotin-dATP using BioNick Labeling Kit (GibcoBRL, Life Technologies Inc., Gaithersburg, MD). Biotinylation is detected with GENE-
- 15   TECT Detection System (CLONTECH Laboratories, Inc. Palo Alto, CA). *In situ* Hybridization is performed on slides using ONCOR Light Hybridization Kit (ONCOR, Gaithersburg, MD) to detect single copy sequences on metaphase chromosomes. Peripheral blood of normal donors is cultured for three days in RPMI 1640 supplemented with 20% FCS, 3% PHA and penicillin/ streptomycin,
- 20   synchronized with  $10^{-7}$  M methotrexate for 17 hours and washed twice with unsupplemented RPMI. Cells are incubated with  $10^{-3}$  M thymidine for 7 hours. The cells are arrested in metaphase after 20 minutes incubation with colcemid (0.5  $\mu$ g/ml) followed by hypotonic lysis in 75 mM KCl for 15 minutes at 37°C. Cell pellets are then spun out and fixed in Carnoy's fixative (3:1 methanol/acetic acid).
- 25   Metaphase spreads are prepared by adding a drop of the suspension onto slides and air dried. Hybridization is performed by adding 100 ng of probe suspended in 10 ml of hybridization mix (50% formamide, 2xSSC, 1% dextran sulfate) with blocking human placental DNA 1  $\mu$ g/ml). Probe mixture is denatured for 10 minutes in 70°C water bath and incubated for 1 hour at 37°C, before placing on a prewarmed (37°C)
- 30   slide, which is previously denatured in 70% formamide/2xSSC at 70°C, and dehydrated in ethanol series, chilled to 4°C.

Slides are incubated for 16 hours at 37°C in a humidified chamber. Slides are  
ashed in 50% formamide/2xSSC for 10 minutes at 41°C and 2xSSC for 7 minutes at  
37°C. Hybridization probe is detected by incubation of the slides with FITC-Avidin  
(ONCOR, Gaithersburg, MD), according to the manufacturer protocol. Chromosomes  
5 are counterstained with propidium iodine suspended in mounting medium. Slides are  
visualized using a Leitz ORTHOPLAN 2-epifluorescence microscope and five  
computer images are taken using Imagenetics Computer and MacIntosh printer.

Once a sequence has been mapped to a precise chromosomal location, the  
physical position of the sequence on the chromosome can be correlated with genetic  
10 map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance  
in Man, which is publicly available on line via computer. The relationship between  
genes and diseases that have been mapped to the same chromosomal region are then  
identified through linkage analysis (Co-Inheritance of Physically Adjacent Genes).

Next, it is necessary to determine the differences in the cDNA or genomic  
15 sequence between affected and unaffected individuals. If a mutation is observed in  
some or all of the affected individuals but not in any normal individuals, then the  
mutation is likely to be the causative agent of the disease.

#### Chromosome assays

20 The sequences of the present invention are also valuable for chromosome  
identification. The sequence is specifically targeted to and can hybridize with a  
particular location on an individual human chromosome. Moreover, there is a current  
need for identifying particular sites on the chromosome. Few chromosome marking  
reagents based on actual sequence data (repeat polymorphisms) are presently available  
25 for marking chromosomal location. The mapping of DNAs to chromosomes  
according to the present invention is an important first step in correlating those  
sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is  
used to clone genomic DNA of a CGRP-RCF gene. This can be accomplished using a  
30 variety of well known techniques and libraries, which generally are available  
commercially. The genomic DNA the is used for *in situ* chromosome mapping using

well known techniques for this purpose. Typically, in accordance with routine procedures for chromosome mapping, some trial and error may be necessary to identify a genomic probe that gives a good *in situ* hybridization signal.

5 In some cases, in addition, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, because primers that span more than one exon in the genomic DNA could complicate the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human  
10 chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments  
15 from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase  
20 chromosomal spread, as described above, can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60. For a review of this technique, see Verma et al., *HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES*, Pergamon Press, New York (1988).

25 With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

### Polypeptide assays

The present invention also relates to a diagnostic assays such as quantitative and diagnostic assays for detecting levels of CGRP-RCF protein in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a  
5 diagnostic assay in accordance with the invention for detecting over-expression of CGRP-RCF protein compared to normal control tissue samples may be used to detect the presence of diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression,  
10 vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like, for example. Assay techniques that can be used to determine levels of a protein, such as an CGRP-RCF protein of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA  
15 assays. Among these ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to CGRP-RCF, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached a detectable reagent such as radioactive, fluorescent or enzymatic reagent, in this example horseradish peroxidase  
20 enzyme.

To carry out an ELISA a sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in  
25 the dish during which time the monoclonal antibodies attach to any CGRP-RCF proteins attached to the polystyrene dish. Unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CGRP-RCF. Unattached reporter antibody is then washed out. Reagents for peroxidase  
30 activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to CGRP-RCF through the primary and secondary antibodies,

produces a colored reaction product. The amount of color developed in a given time period indicates the amount of CGRP-RCF protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

5 A competition assay may be employed wherein antibodies specific to CGRP-RCF attached to a solid support and labeled CGRP-RCF and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of CGRP-RCF in the sample.

### Antibodies

10 The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures  
15 known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a  
20 sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include  
25 the hybridoma technique (Kohler, et al., *Nature* 256:495-497 (1975), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* 4:72 (1983) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985)).

30 Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic

polypeptide products of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or purify the polypeptide of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Thus, among others, antibodies against CGRP-RCF may be employed to inhibit diabetes, migraine, pain and inflammation, Parkinson's disease, acute heart failure, hypotension, urinary retention, osteoporosis, hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychosis, depression, vomiting, benign prostatic hypertrophy, Paget's disease, obesity, cancer, gigantism and the like.

#### **CGRP-RCF binding molecules and assays**

This invention also provides a method for identification of molecules, such as binding molecules, that bind CGRP-RCF/ CGRP receptor. Genes encoding proteins that bind CGRP-RCF/ CGRP receptor, such as binding molecules, can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Such methods are described in many laboratory manuals such as, for instance, Coligan et al., *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

For instance, expression cloning may be employed for this purpose. To this end polyadenylated RNA is prepared from a cell responsive to CGRP-RCF and CGRP receptor, a cDNA library is created from this RNA, the library is divided into pools and the pools are transfected individually into cells that are not responsive to  $\alpha$  - or  $\beta$ -CGRP. The transfected cells then are exposed to labeled  $\alpha$  - or  $\beta$ -CGRP. ( $\alpha$  - or  $\beta$ -CGRP can be labeled by a variety of well-known techniques including standard methods of radio-iodination or inclusion of a recognition site for a site-specific protein kinase.) Following exposure, the cells are fixed and binding of  $\alpha$  - or  $\beta$ -CGRP is determined. These procedures conveniently are carried out on glass slides.

Pools are identified of cDNA that produced  $\alpha$  - or  $\beta$ -CGRP binding cells. Sub-pools are prepared from these positives, transfected into host cells and screened as

described above. Using an iterative sub-pooling and re-screening process, one or more single clones that encode the putative binding molecule, such as a receptor molecule, can be isolated.

Alternatively a labeled ligand can be photoaffinity linked to a cell extract, such  
5 as a membrane or a membrane extract, prepared from cells that express a molecule that it binds, such as a receptor molecule. Cross-linked material is resolved by polyacrylamide gel electrophoresis ("PAGE") and exposed to X-ray film. The labeled complex containing the ligand-receptor can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence  
10 obtained from microsequencing can be used to design unique or degenerate oligonucleotide probes to screen cDNA libraries to identify genes encoding the putative binding molecule.

Polypeptides of the invention also can be used to assess CGRP-RCF binding capacity of CGRP-RCF binding molecules in cells or in cell-free preparations.

15

#### **Agonists and antagonists - assays and molecules**

The CGRP-RCF of the present invention may be employed in a process for screening for compounds which activate (agonists) or inhibit activation (antagonists) of the CGRP receptor or (function of) CGRP-RCF or even CGRP-RCF/ CGRP  
20 receptor system.

In general, such screening procedures involve providing appropriate cells which express the CGRP-RCF/CGRP receptor on the surface thereof. Such cells include cells from mammals, yeast, drosophila or *E. Coli*. In particular, polynucleotides encoding the receptor system of the present invention are employed to  
25 transfect cells to thereby express the CGRP-RCF/CGRP receptor. The expressed polypeptides are then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

One such screening procedure involves the use of melanophores which are transfected to express the CGRP-RCF/CGRP receptor. Such a screening technique is  
30 described in PCT WO 92/01810 published February 6, 1992.

Thus, for example, such assay may be employed for screening for a compound which inhibits activation of the CGRP-RCF/ CGRP receptor by contacting the melanophore cells which encode the polypeptides with both the CGRP receptor ligand and a compound to be screened. Inhibition of the signal generated by the ligand  
5 indicates that a compound is a potential antagonist for the receptor system, i.e., inhibits activation of the CGRP-RCF/ CGRP receptor.

The screen may be employed for determining a compound which activates the receptor system by contacting such cells with compounds to be screened and determining whether such compound generates a signal, i.e., activates the CGRP-RCF/  
10 CGRP receptor.

Other screening techniques include the use of cells which express the CGRP-RCF/ CGRP receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by CGRP-RCF/ CGRP receptor activation, for example, as described in *Science*, 246:181-296 (October 1989). For example,  
15 compounds may be contacted with a cell which expresses the CGRP-RCF/ CGRP receptor and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the CGRP-RCF/ CGRP receptor.

Another such screening technique involves introducing RNAs encoding both  
20 CGRP-RCF and CGRP receptor into *Xenopus* oocytes to transiently express the CGRP-RCF/ CGRP receptor. The oocytes may then be contacted with the CGRP receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium, proton, etc. signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing the CGRP-RCF/ CGRP  
25 receptor in which the receptor system is linked to, for example, a phospholipase C or D or other proteins. As representative examples of such cells, there may be mentioned endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor  
30 system or inhibition of activation of the receptor system from a second signal, such as for example phospholipase or other activated/expressed protein.

Another method involves screening for compounds which inhibit activation of the receptor system of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor system on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNAs encoding both CGRP-RCF and CGRP receptor such that the cell expresses the receptor system on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptor system. If the compound binds to the receptor system as determined by a reduction of labeled ligand which binds to the receptor system, the binding of labeled ligand to the receptor system is inhibited.

Another method involves screening for CGRP-RCF/ CGRP receptor inhibitors by determining inhibition of CGRP-RCF/ CGRP receptor-mediated cAMP and/or adenylate cyclase accumulation. Such a method involves transfecting a eukaryotic cell with the CGRP-RCF/ CGRP receptor system to express the receptor system on the cell surface. The cell is then exposed to potential antagonists in the presence of the receptor system. The amount of cAMP accumulation is then measured. If the potential antagonist binds the receptor system, and thus inhibits ligand binding, the levels of CGRP-RCF/ CGRP receptor mediated cAMP, or adenylate cyclase, activity will be reduced.

The present invention also provides a method for determining whether a ligand, not known to be capable of binding to a CGRP receptor or CGRP-RCF/ CGRP receptor, can bind to such receptor or receptor system which comprises contacting a mammalian cell which expresses CGRP-RCF/ CGRP receptor with the CGRP receptor ligand under conditions permitting binding of ligands to the CGRP-RCF/CGRP receptor system, detecting the presence of a ligand which binds to the receptor and thereby determining whether the ligand binds to the CGRP-RCF/ CGRP receptor. The systems hereinabove described for determining agonists and/or antagonists may also be employed for determining ligands which bind to the receptor system.

Examples of potential CGRP-RCF or CGRP-RCF/CGRP receptor antagonists are also an antibody, or in some cases an oligonucleotide, which binds to the CGRP-RCF or CGRP-RCF/CGRP receptor such that the activity of the polypeptide is prevented.

5        Potential antagonists also include proteins which are closely related to the ligand of the CGRP-RCF/CGRP receptor, i.e. a fragment of the ligand, which have lost biological function and when binding to the receptor system, elicit no response.

A potential antagonist also includes an antisense construct prepared through the use of antisense technology. Antisense technology can be used to control gene  
10       expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is  
15       designed to be complementary to a region of the gene involved in transcription (triple helix -see Lee et al., *Nucl. Acids Res.*, 6:3073 (1979); Cooney et al, *Science*, 241:456 (1988); and Dervan et al., *Science*, 251: 1360 (1991)), thereby preventing transcription and the production of CGRP-RCF. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the CGRP-RCF  
20       (antisense - Okano, *J. Neurochem.*, 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the CGRP-RCF.

Another potential antagonist is a small organic molecule which binds to the  
25       CGRP-RCF or CGRP-RCF/CGRP receptor, making it inaccessible to ligands such that its normal biological activity is prevented.

Potential antagonists also include a soluble form of a CGRP-RCF, e.g. a fragment, which binds to the CGRP receptor ligand and prevents the ligand from interacting with membrane bound CGRP-RCF/CGRP receptor.

30       CGRP-RCF are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate

the CGRP-RCF or CGRP-RCF/ CGRP receptor on the one hand and which can inhibit the function of a CGRP-RCF or CGRP-RCF/ CGRP receptor on the other hand.

In general, agonists for CGRP-RCF or CGRP-RCF/ CGRP receptor are employed for prophylaxis of or for treating Parkinson's disease, acute heart failure,  
5 hypotension, urinary retention.

Antagonists for CGRP-RCF or CGRP-RCF/ CGRP receptor may be employed for prophylaxis of or for treating hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, psychoses, depression, migraine, vomiting, and benign prostatic hyertrophy.

10 The agonists may also be employed to treat osteoporosis since CGRP inhibits osteoclast-mediated bone resorption and stimulates osteogenesis. In this same manner hypercalcemia may be treated. Similarly, the agonists may be employed to treat Paget's Disease.

The agonists may also be employed to stimulate angiogenesis and promote  
15 wound healing via the stimulatory effect of CGRP on endothelial cell proliferation.

The agonists may also be employed to treat obesity since CGRP controls feeding behavior by decreasing appetite and intestinal motility.

The agonists may also be employed to stimulate nerve regeneration since CGRP is a trophic agent in the CNS.

20 The agonists may also be employed to enhance the immune response through increasing vascular permeability.

Agonists may also be employed to inhibit superoxide production. Superoxide production is known in the art to cause cellular damage and lead possibly to diseases such as cancer.

25 The antagonists may also be employed to inhibit CNS pain transmission, to treat chronic inflammation caused by long-lived vasodilation, arthritis, maturity onset diabetes, cardiovascular disorders and to treat migraine headaches.

The antagonists may also be employed to prevent carcinoid tumor of the lung, since an elevated level of CGRP has been found in the lung during this disease.

30 This invention additionally provides a method of treating an abnormal condition related to an excess of CGRP-RCF activity which comprises administering

to a subject the inhibitor compounds (antagonists) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the CGRP-RCF/CGRP receptor, or by inhibiting a second signal, and thereby alleviating the abnormal conditions.

5           The invention also provides a method of treating abnormal conditions related to an under-expression of CGRP-RCF activity which comprises administering to a subject a therapeutically effective amount of a compound which activates the receptor system of the present invention (agonists) as described above in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal conditions.

10

### **Compositions and Kits**

          The soluble form of the CGRP-RCF, and compounds which activate or inhibit such polypeptide receptor system, may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective  
15   amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

          The invention also relates to compositions comprising the polynucleotide or  
20   the polypeptides discussed above. Thus, the polypeptides of the present invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a  
25   therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

          The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned  
30   compositions of the invention.

### Administration

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

The pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific indication or indications. In general, the compositions are administered in an amount of at least about 10 µg/kg body weight. In most cases they will be administered in an amount not in excess of about 8 mg/kg body weight per day. Preferably, in most cases, dose is from about 10 µg/kg to about 1 mg/kg body weight, daily. It will be appreciated that optimum dosage will be determined by standard methods for each treatment modality and indication, taking into account the indication, its severity, route of administration, complicating conditions and the like.

### Gene therapy

The CGRP-RCF polynucleotides, polypeptides, agonists and antagonists that are polypeptides may be employed in accordance with the present invention by expression of such polypeptides *in vivo*, in treatment modalities often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide, such as a DNA or RNA, encoding a polypeptide *ex vivo*, and the engineered cells then can be provided to a patient to be treated with the polypeptide. For example, cells may be engineered *ex vivo* by the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention. Such methods are well-known in the art and their use in the present invention will be apparent from the teachings herein.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by procedures known in the art. For example, a polynucleotide of the invention

may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct then may be isolated and introduced into a packaging cell is transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention.

10 Retroviruses from which the retroviral plasmid vectors herein above mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

Such vectors well include one or more promoters for expressing the polypeptide. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller et al., *Biotechniques* 7:980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, RNA polymerase III, and  $\beta$ -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention will be placed under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter;

inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs herein above described); the  $\beta$ -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, Y-2, Y-AM, PA12, T19-14X, VT-19-17-H2, YCRE, YCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, A., *Human Gene Therapy* 1:5-14 (1990). The vector may be transduced into the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line will generate infectious retroviral vector particles, which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

## EXAMPLES

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplification's, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Certain terms used herein are explained in the foregoing glossary.

All examples are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail.

Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), herein referred to as "Sambrook."

All parts or amounts set out in the following examples are by weight, unless otherwise specified.

Unless otherwise stated size separation of fragments in the examples below is carried out using standard techniques of agarose and polyacrylamide gel electrophoresis ("PAGE") in Sambrook and numerous other references such as, for instance, by Goeddel et al., *Nucleic Acids Res.* 8: 4057 (1980).

Unless described otherwise, ligations are accomplished using standard buffers, incubation temperatures and times, approximately equimolar amounts of the DNA fragments to be ligated and approximately 10 units of T4 DNA ligase ("ligase") per 0.5 µg of DNA.

Example 1 Expression and purification of human CGRP-RCF using bacteria

The DNA sequence encoding human CGRP-RCF in the deposited polynucleotide is digested with specific endonuclease restriction enzyme EcoRI and XhoI to release a fragment encoding the open reading frame of CGRP-RCF and the fragment is purified and ligated into the bacterial expression vector, which is digested with the appropriate enzymes.

The restriction sites are convenient to restriction enzyme sites in the bacterial expression vectors pQE-9 which are used for bacterial expression in these examples. (Qiagen, Inc. Chatsworth, CA. pQE-9 encodes ampicillin antibiotic resistance ("Amp<sup>r</sup>") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), a 6-His tag and restriction enzyme sites.

The human CGRP-RCF DNA and the vector pQE-9 both are digested with EcoRI and XhoI and the digested DNAs then are ligated together. Insertion of the CGRP-RCF DNA into the EcoRI/XhoI restricted vector placed the CGRP-RCF coding region downstream of and operably linked to the vector's IPTG-inducible promoter and in-frame with an initiating AUG appropriately positioned for translation of CGRP-RCF.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan<sup>r</sup>"), is used in carrying out the illustrative example described here. This strain, which is only one of many that are suitable for expressing CGRP-RCF, is available commercially from Qiagen.

Transformants are identified by their ability to grow on LB plates in the presence of ampicillin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA is confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 ug/ml) and kanamycin (25 ug/ml).

The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm ("OD<sup>600</sup>") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from lac repressor sensitive promoters, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation and disrupted, by standard methods. Inclusion bodies are purified from the disrupted cells using routine collection techniques, and protein is solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2X phosphate buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a

further step of chromatography to remove endotoxin. Then, it is sterile filtered. The sterile filtered protein preparation is stored in 2X PBS at a concentration of 95 micrograms per mL.

5    Example 2    Cloning and expression of human CGRP-RCF in a baculovirus expression system

The cDNA sequence encoding the full length human CGRP-RCF protein, in the deposited clone is digested with EcoRI/XhoI to release the CGRP-RCF fragment.

10   The fragment is then inserted into expression vector, which was also digested with the same restriction enzymes.

The vector pRG1 is used to express the CGRP-RCF protein in the baculovirus expression system, using standard methods, such as those described in Summers et al, *A MANUAL OF METHODS FOR BACULOVIRUS VECTORS AND INSECT CELL*  
15   *CULTURE PROCEDURES*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites. The signal peptide of AcMNPV gp67, including the N-terminal methionine, is located just upstream of a BamHI site. The polyadenylation site of the  
20   simian virus 40 ("SV40") is used for efficient polyadenylation. For an easy selection of recombinant virus the beta-galactosidase gene from E.coli is inserted in the same orientation as the polyhedrin promoter and is followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to  
25   generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of pRG1, such as pAc373, pVL941 and pAcIM1 provided, as those of skill in the art will readily appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as  
30   required. Such vectors are described in Luckow et al., *Virology* 170:31-39, among others.

The plasmid is digested with the restriction enzymes EcoRI and XhoI and then is dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is

5 designated herein "V2".

Fragment F2 and the dephosphorylated plasmid V2 are ligated together with T4 DNA ligase. E.coli HB101 cells are transformed with ligation mix and spread on culture plates. Bacteria are identified that contain the plasmid with the human CGRP-RCF gene by digesting DNA from individual colonies using EcoRI and XhoI and then  
10 analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBacCGRP-RCF.

5 µg of the plasmid pBacCGRP-RCF is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by  
15 Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBacCGRP-RCF are mixed in a sterile well of a microtiter plate containing 50 µl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are  
20 added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of  
25 Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of  
30 gal-expressing clones, which produce blue-stained plaques. (A detailed description of

a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after serial dilution, the virus is added to the cells. After appropriate incubation, blue stained plaques are picked with the tip of an Eppendorf pipette. The  
5 agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. A clone containing properly inserted  
10 CGRP-RCF is identified by DNA analysis including restriction mapping and sequencing. This is designated herein as V-CGRP-RCF.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-CGRP-RCF at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours  
15 later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg). 42 hours later, 5 µCi of 35S-methionine and 5 µCi 35S cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and  
20 autoradiography.

### Example 3 Gene therapeutic expression of human CGRP-RCF

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the  
25 tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature overnight. After 24 hours at room temperature, the flask is inverted - the chunks of tissue remain fixed to the bottom of the flask - and fresh media is added (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin). The tissue is  
30 then incubated at 37°C for approximately one week. At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in

culture, a monolayer of fibroblasts emerges. The monolayer is trypsinized and scaled into larger flasks.

A vector for gene therapy is digested with restriction enzymes for cloning a fragment to be expressed. The digested vector is treated with calf intestinal  
5 phosphatase to prevent self-ligation. The dephosphorylated, linear vector is fractionated on an agarose gel and purified.

CGRP-RCF cDNA capable of expressing active CGRP-RCF, is isolated. The ends of the fragment are modified, if necessary, for cloning into the vector. For instance, 5' overhanging may be treated with DNA polymerase to create blunt ends.  
10 3' overhanging ends may be removed using S1 nuclease. Linkers may be ligated to blunt ends with T4 DNA ligase.

Equal quantities of the Moloney murine leukemia virus linear backbone and the CGRP-RCF fragment are mixed together and joined using T4 DNA ligase. The ligation mixture is used to transform *E. coli* and the bacteria are then plated onto agar-  
15 containing kanamycin. Kanamycin phenotype and restriction analysis confirm that the vector has the properly inserted gene.

Packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The vector containing the CGRP-RCF gene is introduced into the  
20 packaging cells by standard techniques. Infectious viral particles containing the CGRP-RCF gene are collected from the packaging cells, which now are called producer cells.

Fresh media is added to the producer cells, and after an appropriate incubation period media is harvested from the plates of confluent producer cells. The media,  
25 containing the infectious viral particles, is filtered through a Millipore filter to remove detached producer cells. The filtered media then is used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the filtered media. Polybrene (Aldrich) may be included in the media to facilitate transduction. After appropriate incubation, the media is removed and replaced with  
30 fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is low, then it is necessary to use a retroviral

vector that has a selectable marker, such as neo or his, to select out transduced cells for expansion.

Engineered fibroblasts then may be injected into rats, either alone or after having been grown to confluence on microcarrier beads, such as cytodex 3 beads. The injected fibroblasts produce CGRP-RCF product, and the biological actions of the protein are conveyed to the host.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

Example 4 *In Vitro* Transcription, Microinjection into *Xenopus laevis* oocytes and Electrophysiology

Capped RNA transcripts were synthesized from linearized pCGRP-RCF or pCGRP-type-I-receptor DNA using T7 RNA polymerase (Stratagene). For microinjection, *X. laevis* oocytes were prepared as previously described (Elshourbagy et al., *J. Biol. Chem.*, 268:3873-3879 (1993)). Stage V-VI oocytes were selected, and the follicular membranes were manually removed. For each experimental group, 6 defolliculated oocytes were coinjected with 50 nl of water containing 20 ng of CGRP-RCF and CGRP type I receptor complementary RNA (cRNA) (Drummond injection apparatus). The injected oocytes were maintained in modified Barth's medium at 18 °C for 48 hrs to allow for protein synthesis and for transport of the receptor protein complex to the cell surface. Electrophysiology was performed using the voltage clamp technique using an oocyte voltage clamp apparatus (Warner Instruments). Oocytes were clamped at -60 mV, and exposed to  $10^{-7}$  M human alpha-CGRP or human Calcitonin (Bachem) and the  $Ca^{+2}$  activated channel activity was recorded in Barth's medium at room temperature.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: CALCITONIN GENE-RELATED PEPTIDE  
RECEPTOR COMPONENT FACTOR (HOUDC44)

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SmithKline Beecham Corporation

(B) STREET: 709 Swedeland Road

(C) CITY: King of Prussia

(D) STATE: PA

(E) COUNTRY: USA

(F) ZIP: 19406-2799

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Han, William T
- (B) REGISTRATION NUMBER: 34,344
- (C) REFERENCE/DOCKET NUMBER: ATG50010

## (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 610-270-5219
- (B) TELEFAX: 610-270-5090
- (C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1450 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE:

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCACGAGCA GCTGTGAAGT GTGAGGTTCT TTGTCTGCTG GCAGCTAGGG GCGACGAGGC	60
GGGACGTCAT GGAAGTGAAG GATGCCAATT CTGCGCTTCT CAGTAACTAC GAGGTATTTC	120
AGTTACTAAC TGATCTGAAA GAGCAGCGTA AAGAAAGTGG AAAGAATAAA CACAGCTCTG	180
GGCAACAGAA CTTGAACACT ATCACCTATG AAACGTTAAA ATACATATCA AAAACACCAT	240
GCAGGCACCA GAGTCCTGAA ATTGTCAGAG AATTTCTCAC AGCATTGAAA AGCCACAAGT	300
TGACCAAAGC TGAGAAGCTC CAGCTGCTGA ACCACCGGCC TGTGACTGCT GTGGAGATCC	360
AGCTGATGGT GGAAGAGAGT GAAGAGCGGC TCACGGAGGA GCAGATTGAA GCTCTTCTCC	420
ACACCGTCAC CAGCATTCTG CCTGCAGAGC CAGAGGCTGA GCAGAAGAAG AATACAAACA	480
GCAATGTGGC AATGGACGAA GAGGACCCAG CATAGAAGAG CACAGCTGGC CCCGGCGTTT	540
CATGAAGTCA GAAGGCCTGG CAGCCATTTT CTGGACGTTG AGAGGATTGT TTATTTGATT	600
TTTATCCTCA TCCCAGCAGG CCTGGCTTTG TGTTAGTTG GGTACATCAC AAAAATAAGT	660

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TAAAAAGAAA TATTTGTGCC TTGGGGAGAA GAAACATGGT GAAAACAGGC TGAGGTTGTC 720
AGGGCAGAGA GCTGAAGGTG GGGACAGTGA CCGCGGACCC CTCTGCGCTT GAAAGATTTC 780
CTCCACGGCC TTTGCCCCAG TTGTGGGGAG GTCTCTGTGC ACAGCGGGGA AAATGCTTGT 840
GTCGCCTTTG GTGGGCCATG TCCTAATTAG TTTCATCTGC TTCCCTGGGA ACTTACTAAG 900
GGGCCCAGAG CACTGTTGGA AGTCTGTTA GAGTCCCCAG AGAGTTACTC TAAGTTAAAA 960
TGAGCCACTG ACCTTGGCTC ACCTTAGAGG AATTTCTCTG AGAACAACAG AGATAAGAAA 1020
AGAACCGGCC TGGCCAATCC TTCAACAGCT CTAGAGCCCC TTTTCTCTGC TGGCAGGGGC 1080
TTTGTTTACC AGCTCACTGT TTAGGCTAAA TGTTAGGGAC CAGATCACTG CAGTTGAAAA 1140
CAGCATCCAG GCTTAGTGAC AGTGGCAGCA GAAACAGTGT TGGCTGCCTT TCTGACCACC 1200
CCACTTTCCT GCCCTGAGAC AGCAGCCCAG GGCAGGTGCT TCATATTAG ACCAGGTAAG 1260
CCTCATTTGC ACAACAGTCA AATTGTTTGT TCCTTTAAAA AGGACACAAT TAGCCTGGCA 1320
CGGTGACTCA TGCTTGTAAAT CCCAGCACTT TGGGAGGGCA AGGCAGGCGG ATCACCTGAG 1380
GTCAGGAGTT TGAGACCAGC CTCACCAACA TGGAAAAACC CCATCTCTAT TAAAAA AAAA 1440
AAAAA AAAA 1450

```

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 148 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (iii) HYPOTHETICAL: NO

## (iv) ANTISENSE: NO

## (v) FRAGMENT TYPE: N-terminal

## (vi) ORIGINAL SOURCE:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Glu Val Lys Asp Ala Asn Ser Ala Leu Leu Ser Asn Tyr Glu Val
 1             5             10             15
Phe Gln Leu Leu Thr Asp Leu Lys Glu Gln Arg Lys Glu Ser Gly Lys
      20             25             30

```

Asn Lys His Ser Ser Gly Gln Gln Asn Leu Asn Thr Ile Thr Tyr Glu  
 35 40 45  
 Thr Leu Lys Tyr Ile Ser Lys Thr Pro Cys Arg His Gln Ser Pro Glu  
 50 55 60  
 Ile Val Arg Glu Phe Leu Thr Ala Leu Lys Ser His Lys Leu Thr Lys  
 65 70 75 80  
 Ala Glu Lys Leu Gln Leu Leu Asn His Arg Pro Val Thr Ala Val Glu  
 85 90 95  
 Ile Gln Leu Met Val Glu Glu Ser Glu Glu Arg Leu Thr Glu Glu Gln  
 100 105 110  
 Ile Glu Ala Leu Leu His Thr Val Thr Ser Ile Leu Pro Ala Glu Pro  
 115 120 125  
 Glu Ala Glu Gln Lys Lys Asn Thr Asn Ser Asn Val Ala Met Asp Glu  
 130 135 140  
 Glu Asp Pro Ala  
 145

What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:
  - 5 (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising amino acids of SEQ ID NO:2;
  - (b) a polynucleotide which by virtue of the redundancy of the genetic code, encodes the same amino acid sequence as amino acids of SEQ ID NO:2;
  - (b) a polynucleotide which is complementary to the polynucleotide of (a)
  - 10 or (b); and
  - (d) a polynucleotide comprising at least 15 bases of the polynucleotide of (a), (b) or (c).
- 15 2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.
3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.
4. The polynucleotide of Claim 2 comprising polynucleotide set forth in SEQ ID NO:1.
- 20 5. The polynucleotide of Claim 2 comprising nucleotide 69 to 512 set forth in SEQ ID NO:1.
6. The polynucleotide of Claim 2 which encodes a polypeptide
- 25 comprising amino acids of SEQ ID NO:2.
7. An isolated polynucleotide comprising a member selected from the group consisting of:
  - 30 (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding the same mature polypeptide expressed by the human cDNA contained in ATCC Deposit No. 98105;

(b) a polynucleotide which by virtue of the redundancy of the genetic code encodes the same mature polypeptide expressed by the human cDNA contained in ATCC No 98105.

- 5 (c) a polynucleotide complementary to the polynucleotide of (a) or (b); and  
(d) a polynucleotide comprising at least 15 bases of the polynucleotide of (a), (b) or (c).

8. A vector comprising the DNA of Claim 2.

10

9. A host cell comprising the vector of Claim 8.

10. A process for producing a polypeptide comprising: expressing from the host cell of Claim 9 a polypeptide encoded by said DNA.

15

11. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of Claim 8 such that the cell expresses the polypeptide encoded by the human cDNA contained in the vector.

20

12. A polypeptide comprising an amino acid sequence which is at least 70% identical to amino acids of SEQ ID NO:2.

13. A polypeptide comprising an amino acid sequence as set forth in SEQ  
25 ID NO:2.

14. An agonist to the polypeptide of claim 12.

15. An antibody against the polypeptide of claim 12.

30

16. An antagonist which inhibits the activity of the polypeptide of claim 12.
17. A method for the treatment of a patient having need of CGRP-RCF comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 12.
18. The method of Claim 17 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.
19. A method for the treatment of a patient having need to inhibit CGRP-RCF polypeptide comprising: administering to the patient a therapeutically effective amount of the antagonist of Claim 16.
20. A process for diagnosing a disease or a susceptibility to a disease related to expression of the polypeptide of claim 12 comprising:  
determining a mutation in the nucleic acid sequence encoding said polypeptide.
21. A diagnostic process comprising:  
analyzing for the presence of the polypeptide of claim 12 in a sample derived from a host.
22. A method for identifying compounds which bind to and activate or inhibit a polypeptide claim 12 comprising:  
contacting a cell expressing on the surface thereof said polypeptide, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

determining whether the compound binds to and activates or inhibits the polypeptide by detecting the presence or absence of a signal generated from the interaction of the compound with the polypeptide.

1 / 1

**FIGURE 1. Human CGRP-Receptor Component Factor Nucleotide and Amino Acid Sequence**

```

1  GGCACGAGCAGCTGTGAAGTGTGAGGTTCTTTGTCTGCTGGCAGCTAGGGGCGACGAGGC. 60
61  GGGACGTCATGGAAGTGAAGGATGCCAATTCTGCGCTTCTCAGTAACACGAGGTATTTC 120
      M E V K D A N S A L L S N Y E V F Q 18
121 AGTTACTAACTGATCTGAAAGAGCAGCGTAAAGAAAGTGGAAAGAATAAACACAGCTCTG 180
19   L L T D L K E Q R K E S G K N K H S S G 38
181 GGCAACAGAACTTGAACACTATCACCTATGAAACGTTAAAATACATATCAAAAACACCAT 240
39   Q Q N L N T I T Y E T L K Y I S K T P C 58
241 GCAGGCACCAGAGTCTCTGAAATTGTCAGAGAATTTCTCACAGCATTGAAAAGCCACAAGT 300
59   R H Q S P E I V R E F L T A L K S H K L 78
301 TGACCAAAGCTGAGAAGCTCCAGCTGCTGAACCAACGGCCTGTGACTGCTGTGGAGATCC 360
79   T K A E K L Q L L N H R P V T A V E I Q 98
361 AGCTGATGGTGGAAGAGAGTGAAGAGCGGCTCACGGAGGAGCAGATTGAAGCTCTTCTCC 420
99   L M V E E S E E R L T E E Q I E A L L H 118
421 ACACCGTCACCAGCATTCTGCCTGCAGAGCCAGAGGCTGAGCAGAAGAAGAATACAAACA 480
119  T V T S I L P A E P E A E Q K K N T N S 138
481 GCAATGTGGCAATGGACGAAGAGGACCCAGCATAGAAGAGCACAGCTGGCCCCGGCGTTT 540
139  N V A M D E E D P A * 158
541 CATGAAGTCAGAAGGCCTGGCAGCCATTTCTTGGACGTTGAGAGGATTGTTTATTTGATT 600
601 TTTATCCTCATCCCAGCAGGCCTGGCTTTGTGGTTAGTTGGGTACATCACAAAATAAGT 660
661 TAAAAAGAAATATTTGTGCCTTGGGGAGAAGAAACATGGTGAAAACAGGCTGAGGTTGTC 720
721 AGGGCAGAGAGCTGAAGGTGGGGACAGTGACCGCGGACCCCTCTGCGCTTGAAAGATTTC 780
781 CTCCACGGCCTTTGCCCCAGTTGTGGGGAGGCTCTCTGTGCACAGCGGGGAAAATGCTTGT 840
841 GTCGCCTTTGGTGGGCCATGTCCTAATTAGTTTCATCTGCTTCCCTGGGAACCTACTAAG 900
901 GGGCCCAGAGCACTGTTGGAAGTCTGGTTAGAGTCCCCAGAGAGTTACTCTAAGTTAAAA 960
961 TGAGCCACTGACCTTGGCTCACCTTAGAGGAATTTCTCGAGAACAACAGAGATAAGAAA 1020
1021 AGAACCGGCCTGGCCAATCCTTCAACAGCTCTAGAGCCCTTTTCTCTGCTGGCAGGGGC 1080
1081 TTTGTTTACCAGCTCACTGTTTAGGCTAAATGTTAGGGACCAGATCACTGCAGTTGAAAA 1140
1141 CAGCATCCAGGCTTAGTGACAGTGGCAGCAGAAACAGTGTGGCTGCCTTTCTGACCACC 1200
1201 CCACTTTCTGCCCTGAGACAGCAGCCAGGGCAGGTGCTTCATATTCAGACCAGGTAAG 1260
1261 CCTCATTTGCACAACAGTCAAATTGTTGTTCCTTTAAAAAGGACACAATTAGCCTGGCA 1320
1321 CGGTGACTCATGCTTGTAATCCCAGCACTTTGGGAGGGCAAGGCAGGCGGATCACCTGAG 1380
1381 GTCAGGAGTTTGAGACCAGCCTACCAACATGGAAAAACCCATCTCTATTAAAAA 1440
1441 AAAAAAAAAA 1450

```

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/12162

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) : C07H 21/04; C12N 15/09, 1/20, 15/00; C12P 21/04 US CL : 536/23.5; 435/320.1, 252.8, 71.2, 172.3 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.5; 435/320.1, 252.8, 71.2, 172.3 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, STN, Biosis, Medline, Scisearch, Embase, Caplus		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Proc. Natl. Acad. Sci., April 1996, Vol. 93, pages 3455-3460, Luebke et al. Identification of a protein that confers calcitonin gene-related peptide responsiveness to oocytes by using a cystic fibrosis transmembrane conductance regulator assay. See Results and Discussion.	1-3, 7-11
Y	The Journal of Biological Chemistry, Aiyar et al. 10 May 1996, Vol. 271, No. 19, pages 11325-11329. A cDNA encoding the calcitonin gene-related peptide type 1 receptor. See Results and Discussion.	1-3, 7-11
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
01 OCTOBER 1996		24 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231		Authorized officer ANDREW WANG <i>[Signature]</i>
Facsimile No. (703) 305-3230		Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/12162

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemical and Biophysical Research Communications, Fluhmann et al., 05 January 1995, Vol. 206, No. 1, pages 341- 347. A human orphan calcitonin receptor-like structure. See Results and Discussion.	1-3, 7-11

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/12162

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 4-6  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
Applicants did not submit a computer readable form of the claimed sequences. See PCT Administrative Instructions, section 513 (c).
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-3, 7-11

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/12162

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I. Claims 1-3 and 7-11 (536/23.5, 435/320.1, 252.8, 71.2, 172.3) are drawn to an isolated cDNA polynucleotide of SEQ. ID. NO. 1 composed of either RNA or DNA that has at least 70% identity with the polypeptide of SEQ. ID. NO. 2 wherein the polynucleotide is contained in a vector that is transformed into a host cell which expresses the polypeptide of SEQ. ID. NO. 2.

Group II. Claims 12-13 (530/350) are drawn to a polypeptide that contains an amino acid sequence that has at least 70% identity with SEQ. ID. NO. 2.

Group III. Claim 14 (530/300, 350) is drawn to an agonist to the polypeptide of SEQ. ID. NO. 2.

Group IV. Claim 15 (530/388.25) is drawn to an antibody against the polypeptide of SEQ. ID. NO. 2.

Group V. Claim 16 (530/300, 350) is drawn to an antagonist to the polypeptide of SEQ. ID. NO. 2 that inhibits its activity.

Group VI. Claims 17-18 (424/130.1, 514/44) are drawn to a method of treatment of a patient having need of CGRP-RCF by administering the DNA to encode the polypeptide of SEQ. ID. NO. 2 or administering the polypeptide alone in a therapeutic amount.

Group VII. Claim 19 (424/130.1) is drawn to a method of inhibiting CGRP-RCF by administering an antagonist to the polypeptide of SEQ. ID. NO. 2.

Group VIII. Claim 20 (436/501) is drawn to a process for diagnosing the disease or susceptibility to a disease related to the expression of the polypeptide of SEQ. ID. NO. 2.

Group IX. Claim 21 (436/501) is drawn to a process of diagnosing the presence of the polynucleotide of SEQ. ID. NO. 2 in a host sample.

Group X. Claim 22 (436/501) is drawn to a method for identifying compounds which bind to and activate or inhibit the polypeptide of SEQ. ID. NO. 2 by contacting a cell expressing said polypeptide with a second component which provides a detectable signal upon binding.

The inventions listed as Groups I-X do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I (claims 1-3 and 7-11) contains a product and a method of use of the product which has the same technical feature that being, a polynucleotide of SEQ. ID. NO. 1. Groups 2-5 are drawn to a polypeptide, an agonist, an antibody, and an antagonist respectively which does not share the same technical feature of group I and groups 6-10 which are drawn to methods. Groups 6-10 are drawn to methods of using the polypeptide or antagonist for treatment or diagnosis which do not share the same technical feature of a polynucleotide of SEQ. ID. NO. 1 in group I.